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Toxicity and Traces of Hg, Pb and Cd in the Hepatopancreas, Gills and Muscles of *Perna viridis* from Jakarta Bay, Indonesia

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ABSTRACT
Heavy metals contamination on the coast of Jakarta Bay has led to the level of pollution and can cause toxicity to organisms living in the sea, i.e., green mussels. Green mussels have the ability to detoxify metals entering their bodies. Their ability to accumulate metals is higher than other aquatic animals. This is due to their sedentary life which prevents them from avoiding the effects of pollution and their high tolerance to certain metals. The high concentration of metal content would be toxic to the cell because metal ions can act as oxidants and bind to organic and protein molecules. The results of the study showed that traces of heavy metals were detected in the hepatopancreas, gills, muscles and gonads organs of the mussels living in the waters of Muara Angke. Lead (Pb) and cadmium (Cd) were found in all four organs, while mercury (Hg) was not detected in the muscles. Traces of Hg and Cd were not detected in hepatopancreas, gills, muscles and gonads of green mussels in Panimbang, while Pb was detected by 0.001 in the male gonads and 0.01 in hepatopancreas. The concentration of Pb in the male gonads are still below the acceptable limit and concentration of Pb in the hepatopancreas is relatively equivalent to the acceptable limit. Metal detection in the organs above shows that the Muara Angke waters tend to be polluted and have an impact on the mussels weight loss as a result of heavy metal toxicity.

Key words: Gills, gonad, hepatopancreas, heavy metals, muscles, toxicity, weight loss

INTRODUCTION

Heavy metals that accumulate in the waters of the northern coast of Java has prevented thousands of fishermen from making their living. Heavy metal contamination in the coast has led to the level of toxicity in biota living in the sea, down to the cellular level. The possible impacts of the toxicity also led to the death of fish, crabs, shrimps (Lestari and Edward, 2004) and even the green mussels cultivated by fishermen and, of course, this affects the ecological balance.

The content of metals in aquatic biota usually continues to increase from time to time. Constant exposures to heavy metals will lead to toxicity in marine biota. In certain circumstances, organisms have the ability to detoxify heavy metals entering their bodies but when it is excessive, it certainly can cause toxicity. Toxicity of heavy metals in the cell caused by the presence of metal ions that act as oxidants and bind to organic molecules or proteins. The metal ions bonds are transferred into the cell cytoplasm and subsequently bind to metallothionein proteins. These bonds will accumulate in the cell. Metal accumulations tend to occur in the liver (Akoto *et al*., 2008). The impact of bioaccumulation of heavy metals in the body of the green mussels may occur directly or indirectly. One immediate effect of heavy metals is the disruption in the body's metabolic processes. Early indications of disruption of metabolic processes in the body is loss of weight (Cordova *et al*., 2011).

This study was conducted to determine traces of heavy metals bioaccumulations in the hepatopancreas, gills, muscles and gonads of green mussels in Panimbang ponds, Muara Angke ponds and Port of Muara Angke, as well as their impacts on the mussels weight loss.

MATERIAL AND METHODS

The sample in this study are the green mussels (Perna viridis) obtained from three places; the port and ponds in Muara Angke, as well as ponds in Panimbang as control. Testing of heavy metals Cd, Hg and Pb was conducted on marine water quality in three locations and the hepatopancreas, gills, muscles and gonads organs, using the AAS method (Atomic Absorption Spectrophotometry) at the Regional Environmental Management Agency (BPLHD) of DKI Jakarta province. Tests were also done to trace heavy metals in the tissues.

Observations of the body weight, length, width and thickness of the mussels were done to test the toxicity of the heavy metals. Measurements were made with a caliper. The parameter for the length of the body was measured from the tip of the anterior to the posterior base, the parameter for the width was measured from the dorsal to the ventral side and the parameter for the height of the shell (thickness of mussel) was measured from the thickness of the shell in closed position.

Statistical analysis: Morphometry and weight data were analyzed with t-test at α 0.05.

RESULTS

Environmental conditions and heavy metals: The study was conducted in three regions, namely the Port of Muara Angke, Muara Angke ponds and Panimbang ponds. Port of Muara Angke (Fig. 1a) is located in urban areas which is very close to the residential area of Jakarta. Environmental condition in Muara Angke tend to be polluted, in which the waters are dirty, smelly, blackish brown, with garbage strewn everywhere and a lot of oil waste on the surface of the waters. It is caused by the hectic human activities in Muara Angke, such as port activities, industry, commerce and recreation. Muara Angke ponds (Fig. 1b) are located amidst Muara Angke waters. The distance between Port of Muara Angke and Muara Angke ponds is about 2.5 kilometers (1.6 miles). Compared to the Port of Muara Angke, the environmental condition of Muara Angke ponds is relatively cleaner, with its light brown water, despite some garbage still found floating on the water surface. Panimbang ponds (Fig. 1c) are located in remote rural areas of Banten, far away from residential areas and there are minimal human activities in its vicinity.

The environmental condition in Panimbang Ponds are relatively clean and unspoiled, characterized by clear, odorless and garbage-free water. Based on direct observation of the environmental conditions above, these three areas are suspected as being polluted. However, the results of heavy metals analysis by BPLHD showed no heavy metals were detected in the water (Table 1).

No heavy metals detected in the water does not mean these three areas are free from them. The highly dynamic nature of water is one of the difficulties in detecting the presence of heavy metals. Therefore, it is necessary to analyze heavy metals in the body of marine biota settling in this water, such as green mussels. The results of the analysis of heavy metals in hepatopancreas, gills, muscles and gonads of the mussels are listed in Table 2.

The results show that heavy metals Hg, Pb and Cd have been accumulated in the male and female gonads of the muscles from the Port of Muara Angke and Muara Angke Ponds. The heavy metals detected in the tissues have already exceeded the acceptable limit. On the contrary, a minimal content of Hg, Pb and Cd has been found in gonads of mussels from Panimbang ponds which confirms that Panimbang ponds are relatively unpolluted.

Concentrations of heavy metals Hg, pb and Cd in the hepatopancreas, muscles and gills of green mussels in Panimbang ponds are undetectable, except for Pb concentrations in the hepatopancreas that was at 0.01. This suggests that the ponds in Panimbang are relatively

Table 1: Results of the analysis of heavy metals in the waters of muara angke, at the port of muara angke and panimbang in 2013, North Jakarta, Indonesia

	Heavy metal			Acceptable Limit (Source: Environment ministerial
Location of study	Hg	Pb	Cd	decree No. 51, 2004)
Muara angke port	*	*	*	Pb = 0.08
Muara angke ponds	*	*	*	Cd = 0.01
Panimbang ponds	*	*	*	Hg = 0.01

(*)Undetected

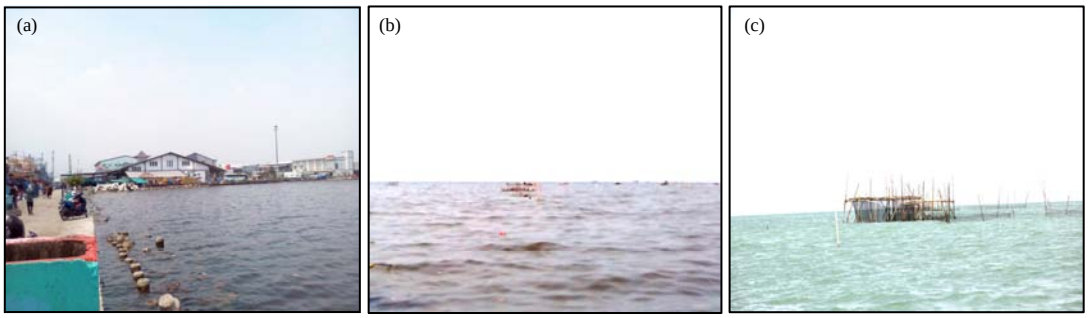

Fig. 1(a-c): Environmental conditions of the waters on the study site (a) Port of Muara Angke (b) Muara angke ponds and (c) Panimbang ponds

Table 2: Concentration of heavy metals Pb, Hg and Cd in the hepatopancreas, gills, muscles and gonads organs of green mussels living in muara angke and panimbang waters, North Jakarta, 2013

	Panimbang ponds (μg g^{-1})					Muara angke ponds (μg g^{-1})					Port of muara angke (μg g^{-1})					Acceptable limit (Source: Environment ministerial decree
Heavy metals	HP	Muscles	Gills	G-♂	G-♀	HP	Muscles	Gills	G-♂	G-♀	HP	Muscles	Gills	G-♂	G-♀	No. 51, 2004)
Hg	*	*	*	*	*	0.03	*	0.01	0.06	0.01	0.06	*	0.03	0.03	0.02	Hg = 0.01
Pb	0.01	*	*	0.001	*	0.02	*	0.04	0.02	0.03	0.04	0.03	0.02	0.04	0.04	Pb = 0.08
Cd	*	*	*	*	*	0.01	0.01	0.01	*	0.02	0.01	0.04	*	0.02	0.02	Cd = 0.01

HP: Hepatopancreas, G: Gonads, *Undetected

Table 3: Data of body weight, length, width and thickness of green mussels (*Perna viridis*) living in the muara angke and panimbang waters, North Jakarta, Indonesia, 2013

	Measurements			
Location	Weight (mg)	Length (mm)	Width (mm)	Thickness (mm)
Port of muara angke	6.20±2.05*	42.85±5.76*	22.76±2.23*	15.70±1.38*
Muara angke ponds	8.14±1.80*	51.17±3.80*	24.77±2.12*	16.51±2.3*
Panimbang ponds	13.89±3.39	62.63±6.08	28.44±2.85	19.92±1.91

*: Significant at $\alpha = 0.05$

uncontaminated. Concentrations of heavy metals Hg, Pb and Cd in hepatopancreas, muscles and gills of mussels in Muara Angke ponds shows that all three heavy metals were found in hepatopancreas and gills, while only Cd was found in the muscles. The heavy metal content is relatively the same and above the established acceptable limit. This suggests that Muara Angke ponds are relatively polluted by heavy metals, such as Hg, Pb and Cd. In general, the organs containing concentrations of Hg, Pb and Cd the most are the hepatopancreas and gills. Most polluted areas based on Table 2 is the port area as opposed to the pond areas.

Data of green mussels' body weight and morphometry: Data from the observations of the weight and morphometry of the green mussels are listed in Table 3. The average weight of green mussels in Port of Muara Angke and Muara Angke ponds is 6.20±2.05 mg and 8.14±1.80 mg, less than the weight of green mussels in Panimbang ponds (3.39±13.89 mg). Statistical test results concluded that the weights are significantly different.

The average body length measurement results of the mussels in Port of Muara Angke and Muara Angke ponds are 42.85±5.76 mm and 51.17±3.80 mm, less than the length of mussels in Panimbang ponds, reaching up to 62.63±6.08 mm. Statistical test results conclude that there is a significance at $\alpha = 0.05$. Similarly, the width and thickness of the green mussels in Port of Muara Angke are on average of 22.76 mm and 15.70±2.23±1.38 and in Muara Angke ponds are 24.77±2.12 and ±2.3 mm 16.51 which is lower than the average width and thickness of the green mussels in Panimbang ponds. From the statistical results, it can be concluded that the difference in width and thickness are significant.

DISCUSSION

The water conditions in the port and ponds of Muara Angke, as well as the ponds in Panimbang showed a difference in terms of environmental condition. The ponds in Panimbang waters are located far from urban areas, in the rural areas of Banten, where the population is still small. Based on direct observation, the condition of the aquatic environment of Panimbang ponds is relatively clear, without strewn garbage and odorless. This condition is due to the lack of people living in the area around Panimbang. Research by Jalius (2008) also explained that heavy metals were undetected in Panimbang waters. Judging from the environmental conditions and direct observation, it is suspected that the water in Panimbang ponds is still uncontaminated. On the other hand, the condition of the pond and port waters in Muara Angke is a lot different, since it is located nearer from urban, more densely populated areas with heavier human activities which greatly affect the surrounding environment. This is indicated by the dirty, garbage-riddled and malodorous water. It is highly suspected that the waters in the port and ponds of Muara Angke are already contaminated, with a condition that gradually worsens from day to day.

Heavy metals analysis by BPLDH reveled that the presence of heavy metals in Panimbang waters were undetected. Judging from the condition of the waters in Panimbang area and supported by the data, it is safe to assume that the area is not contaminated. It is certainly different from Muara Angke waters, where the environmental condition is thought to be leading to severe pollution levels, although based on water analysis by BPLDH, contaminants were not detected (Table 1). This does not mean that the waters in the port and ponds of Muara Angke are free from heavy metals. Heavy metal analysis in this study was also performed on hepatopancreas, gills, muscles and gonads organs of green mussels (Table 2). This is done to prove that the waters of Muara Angke have indeed been contaminated, as it is known that the detection of heavy metals in water is very difficult due to the highly dynamic nature of water, especially sea water, with its constantly moving currents and waves (Rochyatun and Rozak, 2007), causing heavy metals to settle in sediments and would usually be easily found in the bodies of green mussels

which live sedentary lives (Jalius, 2008). Based on the tests of heavy metals content in hepatopancreas, gills, muscles and gonads of the mussels, the presence of heavy metals, especially Pb, Hg and Cd was detected. Therefore it is safe to assume that contaminations have occurred in the areas where Port of Muara Angke and Muara Angke ponds are located.

The presence of heavy metals in hepatopancreas, gills, muscles and gonads of mussels is definitely a concern for other marine lives living around the area, as it has caused toxicity to the green mussels. These animals have a sedentary way of life on the sea bed as filter feeders (Barnes, 1974), causing them to be largely exposed to heavy metals. The constant exposure to heavy metals will have an impact that leads to growth disorders on the biota. Heavy metals entering the water will be removed from the body of water through three processes, namely precipitation, adsorption and absorption by aquatic organisms (Bryan, 1976). Heavy metals in aquatic environments are generally in the form of free ions, organic ion pairs, complex ions and other ionic forms (Palar, 1994).

First entry of heavy metals occurs through the gills. Gills are composed of a layer of flat epithelial directly related to the gills membrane vessels (that allows the heavy metals to be) carried by the bloodstream, spreading them to other organs (Overnell and Sparla, 1990). Although the heavy metals pass along with the water that goes through the gills, it does not mean that there is no accumulation of metals in the gills. This study reveals that there are actually accumulations of Hg, Pb and Cd in the gills of mussels in Muara Angke as opposed to the controls (mussels in Panimbang). This can be explained by the fact that gills contain mucus which main constituent is glycoprotein. Metals will be bound to the metallothionein protein (a glycoprotein) which main constituent is cysteine. Thus, metal accumulation in the gills is probable. Heavy metals are absorbed by the body of organisms through a number of cell membranes, composing of lipid and protein membranes. Entries of heavy metals can occur through diffusions or active transports, depending on the form of their compounds. In the body, metals will form complex bonds with ligands. Metal ions can bind to groups of hydroxyl, carboxyl, sulfhydryl and amino acids in protein. Metal ions more effectively bound to sulfhydryl groups, such as cysteine having a nitrogen group. Sulfur and nitrogen group is an active group of an enzyme. Therefore, the action mechanism of heavy metals always attacks the active group of an enzyme which is the sulfide bond.

Another organ where heavy metals are accumulated most is the hepatopancreas. This organ is rich in sulfide bonds, as well as digestive enzymes needed for the detoxification process. Therefore, the heavy metals test on hepatopancreas resulted in a very high number, 0.06 (Table 2) compared to the other organs; gills, muscles and gonads (Prakash *et al*., 1994). Ranks of heavy metals accumulation from the highest to the lowest is in the hepatopancreas, gills, gonads and muscles. The ability of hepatopancreas to accumulate heavy metals is due to its being equipped with very complex mucus and has about 80% ability to accumulate heavy metals, related to sulfhydryl groups within lysosomes. Heavy metals enter the organs by way of active diffusion through endocytosis vesicles, hence accumulated in lysosomes (Jalius, 2008).

The result of the study by Putri *et al*. (2012), was that the waters of Muara Kamal is already polluted by heavy metals exceeding the acceptable limits. The results of the study stated that the contents of heavy metals in the body tissues of green mussels in Muara Kamal were found at 0.92-1.425 (ug g^{-1}) for Pb, 0.46-0743 (g g^{-1}) for Cd and 0.0017-0.012 (g g^{-1}) for Hg. The study done in Muara Angke reveals that the contents of Hg, Pb and Cd in the organs of green mussels there are relatively higher compared to those in Muara Kamal. Therefore, the relatively high concentration is something to be alert about, for heavy metals dissolved in water columns and inside the body (of organisms) at a certain concentration can become a source of poison to aquatic life (Palar, 1994). Toxicity caused by one type of heavy metals may differ from one aquatic biota to another but the destruction of a group can break a link in the chain of life.

Even in small amounts, heavy metals can kill living organisms, such as the green mussels. The process begins by the accumulation of heavy metals which in the long run may exceed the tolerance of the mussels' bodies. The impacts of heavy metals bioaccumulation in the mussels' bodies can be either direct or indirect. One of the direct impacts of heavy metals bioaccumulation in the body of an organism is the disruptions in the metabolic processes in the body. Early indications of such disruptions is the weight loss, as a result of heavy metal toxicity. This condition interrupts the growth of the green mussels, resulting in smaller than normal size and reduced distribution (Cordova *et al*., 2011) and can further cause death to the mussels.

CONCLUSION

Due to the accumulated heavy metals in their bodies, the condition of green mussels in Jakarta Bay is worsening every year.

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